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Risk-factors and predictors of mortality in patients colonised with vancomycin-resistant enterococci

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ABSTRACT

Vancomycin-resistant enterococci (VRE) have emerged as significant nosocomial pathogens. A hospital-wide prevalence study was performed to identify cases with VRE faecal colonisation. A case-control study using two randomly selected VRE-negative controls for each positive case was performed to assess risk-factors for VRE colonisation by univariate and multivariate analysis. VRE faecal colonisation was documented in 53 (14.3%) of 370 patients screened. Previous exposure to anti-anaerobic agents, as well as quinolones, was associated with VRE colonisation ($p < 0.05$). The presence of an invasive device (OR 4.8, p 0.003) and the duration of any antimicrobial treatment before VRE isolation (OR 1.2, $p < 0.001$) predicted VRE colonisation in multivariate models. The crude mortality rate for patients with VRE colonisation was 24.5%, but VRE colonisation was not an independent predictor of mortality in these patients. These results suggest that an active surveillance programme focusing on specific patient groups may help in the identification of VRE-colonised patients. Promptly implemented infection control strategies targeting these groups should help to combat the rising incidence of VRE.

Keywords Colonisation, enterococci, mortality, risk-factors, vancomycin resistance, vancomycin-resistant enterococci

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INTRODUCTION

Enterococci with acquired, plasmid-mediated, high-level resistance to glycopeptide antimicrobial agents have been implicated increasingly in nosocomial outbreaks that result in high morbidity and mortality [1–4]. In 2003, 28.5% of enterococcal isolates from intensive care units (ICUs) that were reported to the National Nosocomial Infections Surveillance system in the USA were resistant to vancomycin [5]. Strains of *Enterococcus faecium* predominate among vancomycin-resistant enterococci (VRE), with an average of 50% showing resistance to vancomycin [6,7]. Although *Enterococcus faecalis* is the most prevalent of all enterococci causing infections, more recent data show an increase in the proportion of infections caused by *E. faecium*, ranging from 15% to 20% [7]. An increasing prevalence of vancomycin-resistant *E. faecium* isolates belonging to the epidemic and virulent hospital-adapted clonal complex-17 (CC-17) among bloodstream isolates has been documented in both North America and Europe as part of the SENTRY antimicrobial surveillance programme [8]. Reported VRE colonisation rates among hospitalised patients vary widely, ranging from 1.5% to 32% [9–14], while the prevalence of VRE among non-hospitalised patients is 1–3.5%, usually involving non-epidemic isolates [11,14,15].

Newly detected VRE may represent either acquisition of resistant organisms or genes (nosocomial transmission) or expansion of pre-existing, but undetected, colonisation with VRE following heavy exposure to antimicrobial agents [16], rather than de-novo emergence of resistance [17]. Thus, the likelihood of nosocomial VRE

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may vary according to the 'colonisation pressure', i.e., the degree of endemicity of VRE in a specific location, exposure to contaminated equipment, and proximity to a VRE carrier, as well as the 'time at risk', i.e., the duration of hospitalisation [18]. However, it has been difficult to differentiate among the factors associated with amplification of previously undetectable colonisation and those associated with nosocomial spread of VRE.

In February 2005, several isolates of VRE from clinical specimens were noted in the University General Hospital Attikon (Athens, Greece). These specimens were derived from blood, urine, a central venous catheter and a surgical wound swab. Immediate therapeutic and infection control measures were implemented for the identified cases. In order to achieve a thorough understanding of the epidemiology of VRE in the hospital, a hospital-wide prevalence study was conducted to identify patients who were colonised with VRE. A case-control comparison with non-colonised patients was then performed to evaluate the risk-factors for colonisation.

MATERIALS AND METHODS

Study site and ethical approval

The study was undertaken between 20 April and 30 May 2005 in Attikon General Hospital, a 330-bed tertiary university hospital in Athens, Greece, that admits *c.* 19 000 patients annually. All hospitalised patients were surveyed by obtaining rectal swab cultures at specified intervals during the study period. The ICU was not included in the study, as it was already undergoing active surveillance for VRE and multiresistant Gram-negative bacteria. The study protocol was approved by the hospital's Ethical Committee. As this measure was considered to be a relatively minor expansion of the existing infection control programme for screening patients, only verbal patient consent was obtained [19].

Protocol for culture of VRE

A rectal swab was collected from every consenting hospitalised patient in three consecutive surveys, each of which lasted 5 days (an entire working week). For logistical reasons, the period between the first two surveys was 25 days, and that between the second and third surveys was 10 days. The swabs were transported to the microbiology laboratory for selective culture of glycopeptide-resistant enterococci. Patients found to be colonised or infected with VRE were placed under contact isolation precautions according to CDC guidelines [20]. Patients who were colonised with *Enterococcus gallinarum* or *Enterococcus casseliflavus* were excluded from the study, as these species demonstrate low-level, intrinsic, chromosomal, non-transferable vancomycin resistance [21].

Culture, identification and susceptibility testing

Swabs were inoculated on BBL Enterococcosel agar plates containing vancomycin 6 mg/L (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). All plates were incubated aerobically at 37°C for 48 h. One or more colonies from each plate that morphologically resembled enterococci (i.e., with a dark brown halo) were initially identified according to Gram's stain, growth in NaCl 6.5% w/v broth and bile aesculin hydrolysis. Species identification was performed using the Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks, MD, USA) according to the manufacturer's instructions. Confirmatory identification of *E. faecium* was by multiplex PCR based on specific detection of *ddl* genes encoding the D-alanine–D-alanine ligase. MICs of antimicrobial agents were determined using the broth microdilution method. Isolates with vancomycin MICs <4 mg/L were classified as vancomycin-susceptible, isolates with vancomycin MICs of 4–16 mg/L were classified as intermediate, and isolates with vancomycin MICs ≥32 mg/L were classified as VRE [22]. Susceptibility to vancomycin and teicoplanin was also assessed using Etests (AB Biodisk, Dalvagen, Sweden).

Pulsed-field gel electrophoresis (PFGE) typing

Clonal relationships were assessed using PFGE of *Sma*I-digested fragments of genomic DNA, performed according to standard PFGE methods. Macrorestriction fragments were compared visually for similarities and clonal groups were determined according to established criteria [23]; i.e., isolates were considered to be indistinguishable, or closely related, if there were three or fewer band fragment differences when compared to the common (modal) type.

Amplification of resistance genes

A multiplex PCR was used with eight different pairs of primers specific for the identification of *E. faecium* and *E. faecalis* (16S rRNA) and the *vanA*, *vanB*, *vanC*, *vanD*, *vanG* and *vanE* glycopeptide resistance genotypes, according to the protocol described by Depardieu *et al.* [24]. *E. faecalis* ATCC 51299 (*vanB*) and *E. faecalis* (*vanA*) were used as control strains.

Epidemiological investigation

The study identified patients colonised with VRE (cases). For each patient colonised with VRE, two patients who failed to yield VRE isolates in surveillance cultures were randomly selected as controls from among the entire population surveyed, with the aid of random number generator software. Data registered for each patient included demographics, previous hospitalisation, underlying medical conditions, immunodeficiency status (e.g., use of immunosuppressive agents such as glucocorticoids, cyclosporin and anti-cancer chemotherapy, or infection with human immunodeficiency virus), hospital ward at time of sampling, presence of an invasive device (central venous or urinary catheter), exposure to an antimicrobial agent (both current and previous, i.e., within the previous 6 months), and duration of hospital stay before study entry.

Statistical analysis

Results were expressed as median and inter-quartile range. Chi-squared and Fisher's exact tests were used for categorical variables. Non-parametric statistical tests (Mann–Whitney's *U*-test or Wilcoxon's signed-rank test) were used to compare continuous variables, e.g., age, duration of hospital stay and duration of antimicrobial use between cases and controls. Normally distributed variables were compared using analysis of variance. Spearman's correlation coefficient was used to assess the relationship between continuously distributed variables, e.g., age, length of hospital stay and duration of antimicrobial use. General logistic regression analysis to identify predictors of colonisation with VRE was performed for variables associated with VRE colonisation during univariate analysis ($p < 0.10$), together with selected variables that have been shown to be significant in previous studies (e.g., chronic renal disease, diabetes mellitus). Interactions of specific terms were also studied. All statistical tests used were two-sided, with $p < 0.05$ considered to be statistically significant. SPSS v.10.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for data analysis.

RESULTS

Prevalence of VRE colonisation

During the 40-day period of the study, 448 patients from the entire hospital (except the ICU) were eligible for the survey. Forty (8.9%) patients were away from the ward at the time of screening and 38 (8.5%) patients refused consent. From the remaining 370 (82.5%) patients, 465 rectal swab specimens were collected, of which 73 (15.7%) from 53 (14.3%) patients were VRE-positive. The prevalence of VRE carriage in the three surveys was 19.7%, 14.4% and 9.5%, respectively. All isolates were identified as *E. faecium* carrying the *vanA* gene. In addition, five clinical isolates (all from patients in the haematological ward) were identified as linezolid-resistant *E. faecium* (vancomycin- and linezolid-resistant enterococci (VLRE)), with a linezolid MIC of 12 mg/L. These were the first VLRE isolates identified in this hospital. None of these five patients was receiving therapy with linezolid.

PFGE analysis

Eight unique PFGE restriction profiles were identified. Two clonal types (A and B) were the most frequent, containing 29 and 25 isolates, respectively. No association of the most frequent clonal types with a specific hospital location was found.

Patients' characteristics

Clinical features of patients with ($n = 53$) or without ($n = 106$) VRE rectal colonisation are summarised in Table 1. The two groups did not differ with respect to age or gender. Patients colonised with VRE at the time of the initial culture had a significantly longer current hospital stay as compared to non-colonised patients. Univariate analysis showed that VRE-positive patients were more likely than controls to suffer from a haematological malignancy or immunodeficiency, whereas the presence of a solid tumour, diabetes mellitus or renal disease was not associated with VRE colonisation (Table 1).

Antimicrobial use

Exposure to antimicrobial agents before the initial positive screen for VRE is also shown in Table 1. VRE-positive patients were more likely to have been treated with intravenous vancomycin, piperacillin–tazobactam, carbapenems, anti-anaerobic agents, quinolones or any antibiotic (all grouped together), both during the current period of hospitalisation and within the previous 6 months. Preceding exposure to aminopenicillins, aminoglycosides and cephalosporins was similar for the two groups. VRE carriers had a longer duration of any antimicrobial use in the current period of hospitalisation and before study entry.

Clinical outcome

In terms of clinical outcome, none of the patients developed a VRE infection during the current period of hospitalisation. VRE-positive patients had longer total periods of hospitalisation than did VRE-negative patients. This difference was observed in the duration of hospitalisation before the initial culture as well as afterwards.

Eighteen (11.3%) of 159 patients included in the study died. VRE-colonised patients were more likely to die in the hospital, but none of the deaths was attributable to a VRE infection. The crude mortality rate for colonised patients was 24.5% (13/53) as compared to 4.7% (5/106) for patients who were not colonised with VRE (OR 6.6, 95% CI 2.2–19.6, $p < 0.001$). Univariate analysis showed that patients who died were older (p 0.001), more likely to have a malignancy

Table 1. Comparison by univariate analysis of the clinical features, antimicrobial use and outcomes of patients with or without vancomycin-resistant enterococci (VRE) rectal colonisation

Characteristic	VRE-positive (n = 53) (%)	VRE-negative (n = 106) (%)	p	OR (95% CI)
Gender, male (%)	31/53 (58.5)	47/106 (43.9)	0.9	
Age, years[a]	67 (52.5–77)	69 (52.8–78)	0.7	
Hospitalisation in the preceding 6 months	40/50 (80)	55/104 (52.9)	0.001	3.6 (1.6–7.9)
Hospitalisation in intensive care unit in the preceding 6 months	5/50 (10)	1/105 (0.95)	0.01	11.6 (1.3–101.7)
Co-morbidities				
Haematological malignancy	18/53 (34)	12/106 (11.3)	0.001	4.0 (1.8–9.2)
Solid tumour	10/53 (18.7)	24/106 (22.6)	0.7	
Immunodeficiency	27/53 (50.9)	26/106 (24.5)	0.001	3.2 (1.6–7.9)
Diabetes mellitus	14/53 (26.4)	25/105 (23.8)	0.8	
Renal disease	7/53 (13.2)	12/105 (11.4)	0.8	
Presence of an invasive device	33/49 (67.3)	22/102 (21.6)	<0.001	7.5 (3.5–16)
Hospitalisation in the two wards for patients with haematological malignancy	29/53 (54.7)	32/106 (30.2)	0.003	
Hospitalisation days before culture[a]	18 (6–30)	5 (3–11)	<0.001	
Hospitalisation for ≥10 days before culture	35/53 (66)	30/106 (28.3)	<0.001	4.9 (2.4–10)
Hospitalisation days after culture[a]	11 (7–22.5)	6 (1–12)	0.001	
Length of total hospitalisation[a]	34 (18–48)	14 (8–26)	<0.001	
Crude mortality	13/53 (25)	5/106 (4.7)	<0.001	6.6 (2.2–19.6)
Antimicrobial use				
Previous use of antibiotics (within preceding 6 months)	35/47 (74.5)	40/90 (44.4)	0.001	3.6 (1.7–7.9)
Current use of any antibiotics (within the preceding 2 days)	41/53 (77.3)	32/106 (30.2)	<0.001	7.9 (3.7–17)
Days of antimicrobial treatment before culture[a]	8 (2.5–20)	0 (0–2)	<0.001	
Vancomycin	13/53 (24.5)	1/106 (0.9)	<0.001	34 (4.3–269)
Ampicillin–sulbactam	3/53 (5.7)	7/106 (6.6)	1.0	
Piperacillin–tazobactam	18/53 (34)	9/106 (8.5)	<0.001	5.6 (2.3–13.5)
Clindamycin	4/53 (7.5)	1/106 (0.9)	0.04	
Metronidazole	2/53 (3.8)	8/106 (7.5)	0.5	
Anti-anaerobic agents[b]	32/53 (60.4)	20/106 (18.9)	<0.001	6.6 (3.1–13.7)
Cephalosporins, second generation	3/53 (5.7)	7/106 (6.6)	1.0	
Cephalosporins, third and fourth generations	3/53 (5.7)	3/106 (2.8)	0.4	
Quinolones	14/53 (26.4)	6/106 (5.7)	0.001	6 (2.1–16.7)
Aminoglycosides	3/53 (5.7)	2/106 (1.9)	0.3	
Carbapenems	11/53 (20.7)	1/106 (0.9)	<0.001	27.5 (3.4–220)

[a]Continuous variables expressed as median (inter-quartile range).
[b]Piperacillin–tazobactam, ampicillin–sulbactam, amoxycillin–clavulanate, cefoxitin, imipenem–cilastatin, meropenem, metronidazole or clindamycin.

(OR 3.6, 95% CI 1.3–10.3, p 0.02) and, more specifically, a haematological malignancy (OR 3.3, 95% CI 1.1–9.3, p 0.05). In addition, they were more likely to have an invasive device in place (OR 7.9, 95% CI 2.4–25.3, p <0.001), a longer duration of hospitalisation (for a 10-day stay, OR 3.3, 95% CI 1.2–9.4, p 0.02), and to have had more prolonged use of any antibiotic (p <0.001).

Multivariate analysis

Logistic regression analysis, after adjustment for age, gender, duration of hospital stay before the positive culture result, hospitalisation in specific wards, presence of immunodeficiency, and haematological malignancy, revealed that the presence of an invasive device and the duration of antimicrobial treatment before VRE isolation (Fig. 1, Table 2) were the most important predictors of VRE colonisation. Regarding exposure to specific antimicrobial agents, other adjusted multivariate models have revealed that intravenous treatment with anti-anaerobic agents, as well as quinolone use, are associated significantly with VRE colonisation. In a multivariate model, the most important predictors of death in the study group were increasing age and the diagnosis of a

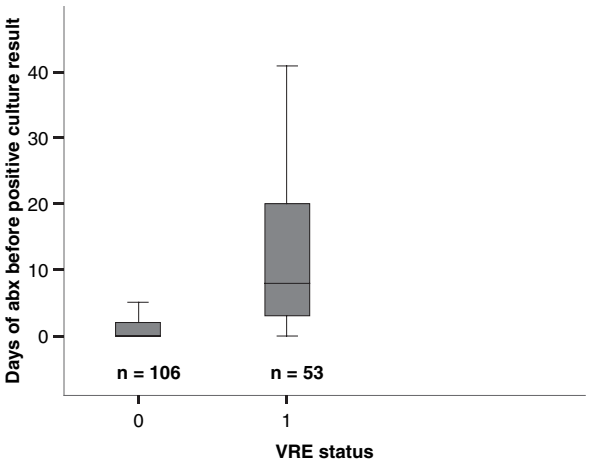

Fig. 1. Days of antibiotic treatment (abx) before sampling for patients positive for colonisation with vancomycin-resistant enterococci, shown in column 1, and controls, shown in column 0.

Table 2. Multivariate analysis of risk-factors for colonisation with vancomycin-resistant enterococci (VRE) and death

Variable	OR (95% CI)	p
Risk of VRE colonisation		
Any invasive device	4.8 (1.7–13.5)	0.003
Duration of any antimicrobial treatment before VRE isolation[a]	1.2 (1.1–1.3)	<0.001
Specific antimicrobial exposure[b]		
Anti-anaerobic agents	4.8 (1.9–12)	0.001
Quinolone	4.1 (1.1–15.3)	0.03
Risk of in-hospital death		
Increasing age[c]	1.08 (1.02–1.15)	0.009
Malignancy	8.2 (1.2–53.8)	0.03
VRE colonisation	3.1 (0.6–15.1)	0.2
Chronic renal failure	6.4 (0.8–53.5)	0.09

[a]OR expressed per additional day of treatment.
[b]In models adjusting for age, gender, immunodeficiency, duration of hospital stay before the positive culture result, and presence of a haematological malignancy or an invasive device.
[c]OR expressed per additional year.

malignancy, but not VRE colonisation, while the presence of chronic renal failure approached, but did not reach, statistical significance (Table 2).

DISCUSSION

This study investigated the clinical characteristics, antibiotic use, microbiological factors and clinical outcome of patients colonised with VRE. The overall prevalence of VRE of 14.4% was somewhat higher than those reported previously by other investigators in Europe (1.5–8.6%) [11–13] or specifically in Greece (3.9–7.5%) [25,26]. The PFGE results, showing that most isolates belonged to two major clones, with the remainder belonging to six additional clones, suggest an endemic situation, combined with an acute nosocomial outbreak. The two major clones were not located in specific wards, but were disseminated throughout the hospital. In multivariate analysis, two major variables were found to be associated with VRE colonisation, i.e., the presence of an invasive device and the duration of any antimicrobial treatment before VRE isolation. Intravenous treatment with anti-anaerobic agents, and the use of quinolones, were also found to be related positively to VRE colonisation.

The association between VRE colonisation or infection and certain underlying medical conditions, e.g., diabetes mellitus, chronic renal failure, malignancies and transplantation, is well-known [4,9,16,27,28]. In the present study, haematology patients were identified as a group at increased risk for VRE colonisation in univariate analysis. However, hospitalisation in wards where the vast majority of patients suffer from a haematological disease was also associated with VRE colonisation. This association is further confounded by the fact that most patients in haematology wards are heavily exposed to broad-spectrum antibiotics, resulting in higher colonisation pressure and enhanced spread of VRE [28]. Matar *et al.* [9] have reported that a significant proportion (29%) of VRE-colonised patients with haematological malignancy develop a subsequent VRE bacteraemia, or another infection (32%), mostly while neutropenic (71%).

The presence of an invasive device has been identified previously as a strong clinical risk-factor for VRE invasive infections [28]. It is unclear whether catheters serve as the actual conduit through which VRE infection is acquired, or whether they are just markers of debilitation, prolonged hospitalisation and severe co-morbidities.

The possibility that preceding antimicrobial treatment is a risk-factor for nosocomial VRE has been explored in numerous studies of both colonised and infected patients, with conflicting results [15]. The most important agents reported are vancomycin [4,10,28–30], cephalosporins [10,16,18,29,31,32] and antimicrobial agents with an anti-anaerobic spectrum [4,16,32–34]. The exact role of vancomycin may need further elucidation. It has been reported that ongoing vancomycin use maintains an intestinal environment favourable to VRE growth, thus increasing the likelihood of VRE entry into the bloodstream [30]. Preceding treatment with vancomycin was more common in VRE-positive patients in the present study, but this association did not reach statistical significance in multivariate models. A meta-analysis by Carmeli *et al.* [35] revealed that the reported strong association between vancomycin treatment and hospital-acquired VRE is the result of reference group selection and of publication bias confounded by a longer duration of hospitalisation. The present study, which accounted for these factors, revealed only a small and non-significant association, which is in concordance with other studies [4,15,31,33]. Glycopeptide use may promote the possibility of a VRE carrier becoming a transmitter, rather than increasing the risk of a non-carrier becoming colonised [15,36].

The present study revealed a significant association between exposure to agents with anti-anaerobic activity and VRE colonisation. These

agents may increase VRE colonisation in the lower gastrointestinal tract [4] through suppression of gastrointestinal anaerobic flora [16]. A higher risk of bacteraemia has been observed in such patients [37], and limiting the use of anti-anaerobic agents may therefore help to decrease the spread of VRE [21].

The relationship between exposure to fluoroquinolones and VRE colonisation or infection is unclear. A significant association was revealed between exposure to fluoroquinolones and VRE colonisation in some, but not all, of the multivariate models examined (data not shown). A meta-analysis of ten studies revealed a possible role of quinolones in the nosocomial epidemiology of VRE [38]. Other studies have demonstrated the importance of the duration of exposure to quinolones [15] and/or their use as a prophylaxis regimen [39].

The failure of the present study to detect the well-known association between exposure to third-generation cephalosporins and VRE colonisation [10,16,18,29,31,33] may be related to the limited use of these drugs in this institution. Nevertheless, the data confirm the importance of the length of antimicrobial exposure, rather than the use of specific antimicrobial agents.

Other factors may also be important. In univariate analyses, hospitalisation in a medical ward or admission to the ICU during the preceding 6 months was associated with VRE colonisation. These results either reflect an increased severity of illness in the population studied or may represent previous VRE acquisition in high-risk environments [27,40].

Colonisation with VRE may lead to invasive infections, but no such events were observed during this study, despite the fact that all patients were followed until discharge. It has been shown that VRE colonisation is universally present in patients developing bacteraemia [4], and a study of immunodeficient patients revealed a high negative predictive value (99.9%) and a positive predictive value of 29.3% for VRE colonisation with the development of bacteraemia [9]. VRE bacteraemia may emerge in the setting of prolonged gastrointestinal colonisation as an isolated breakthrough event under heavy antimicrobial treatment.

Invasive VRE infections have been associated with high morbidity and mortality rates [1–4]. Previous studies have attributed the higher crude mortality rate observed in patients infected with VRE to the underlying severity of illness [28,39]. In the present study, prolonged hospitalisation and high crude mortality rates were related to VRE isolation in univariate analyses. However, as in other studies, VRE colonisation was not an independent risk-factor for all-cause mortality [10,33]. As expected, the main predictors of death were underlying malignancy and increasing age.

The present study was limited by its cross-sectional design and the specific setting studied. For example, the risk of colonisation could not be assessed in patients who were initially free of VRE [19], and the results may have been affected by the individual practices of physicians in the hospital. The observed results apply to the individual risk of VRE colonisation and do not consider the role of transmission of VRE from other patients, most likely via the hands of healthcare workers or contamination of environmental surfaces. It has been reported that poor infection control practices, admission of patients who are already colonised, antibiotic use and prolonged hospitalisation all contribute to the spread of VRE in hospitals [37].

Haematological patients with invasive devices, who are treated with antibiotics for a prolonged period in the same ward, may have served as a reservoir for VRE spread. The importance of proximity in time and space to culture-positive patients for transmission of VRE has been shown previously [34], but was not analysed in the present study. Byers *et al.* [34] revealed that proximity to non-isolated, colonised patients was an important risk-factor for acquisition of VRE, and also suggested that antibiotic exposure is an important, but insufficient, precondition for developing a positive VRE culture among patients who have not previously been exposed to VRE. Although a careful attempt was made to adjust for most confounders, residual confounding could not be taken into account.

Enterococci have now emerged as significant urinary tract, surgical wound and bloodstream pathogens. The discovery of a globally dispersed clonal lineage of the virulent hospital-adapted clone of *E. faecium* (CC-17) has provided an explanation for the rapid spread of resistant strains within the hospital environment. The acquisition of ampicillin resistance and the putative *esp* pathogenicity island by *E. faecium* has improved the relative fitness of this species in

hospital environments, thereby facilitating transmission and leading to nosocomial outbreaks [41]. However, 10- to 20-fold more patients are colonised than are infected [36], and the most interesting studies are those that investigate the epidemiology of colonisation with VRE [42], with the expectation that VRE colonisation will eventually lead to infections [32]. In one study, 14% of colonised patients eventually yielded a positive clinical culture a median of 15 days after a positive surveillance culture [43]. Identification of the patient groups at risk of colonisation, and the implementation of aggressive screening and infection control measures, are necessary [43,44] and effective [27,45]. Infection control measures designed to prevent nosocomial spread of VRE remain important [20,45,46], although incomplete compliance with these measures is a problem [4,28,40].

The present study suggested that patients with an invasive device and heavy antimicrobial exposure constituted the group at highest risk of VRE colonisation. Although not identified in this study, such colonisation may eventually account for increased rates of clinical infection [4,9]. Thus an active surveillance programme was instituted for early detection of VRE-colonised patients, specifically targeting the groups at highest risk in the hospital. An effort was also made to control antibiotic use more effectively and to strictly implement a hand hygiene policy. The drop in prevalence in the three consecutive surveys may indicate a fluctuation with time, although specific control measures were implemented after the first screening period. These efforts might have biased some of the associations observed, although a hospital with continuous movement of patients would expect some of the factors inherent in VRE colonisation to remain unchanged (i.e., presence of a malignancy or an invasive device). Long-term evaluation of these measures is ongoing and should help to control future outbreaks of clinical infection with this multiresistant pathogen. Studies with similar design may be used to devise targeted, and perhaps more effective, control strategies for VRE.

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